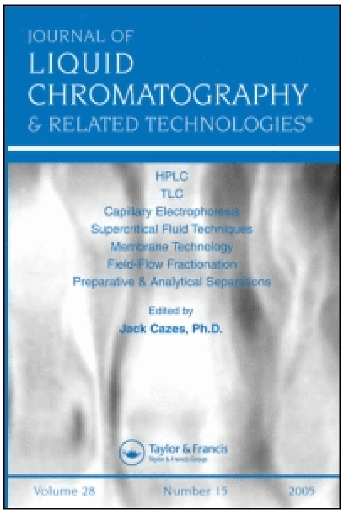

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ELECTRONIC SCANNING FOR FLAT BED DENSITOMETRY

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ABSTRACT

The paper discusses the advantages and disadvantages of replacing the traditional optical scanning methods used in densitometers for the evaluation of 1D and 2D flat bed separations (chromatography as well as electrophoresis) by electronic devices. The main advantage expected from such a replacement are lower hardware cost and much higher data acquisition speed. Disadvantages are spectral limitations and a more difficult optical design.

Electronic scanning can be performed by vacuum tubes (vidicons) and solid state devices. The trend goes to increased use of the latter. Solid state sensors can in turn be divided into line and matrix sensors. Only devices in large scale integrated design are considered. The relative advantages and disadvantages of the various types of sensors are briefly discussed also in comparison to optical scanning. Briefly mentioned are high resolution laser scanning and pyroelectric arrays with flat spectral response. In the final paragraph are outlined specific problems associated with sampling, analog to digital conversion and storage for the high speed, high volume data flow produced by electronic scanning.

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INTRODUCTION

Optical densitometry is already many years the standard method for the quantification and, to a lesser extent, identification of the components of complex solutions, which were separated by thin layer chromatography (TLC) or electrophoresis (EP) on sheet-shaped flat substrates. Both principal separating methods have one-dimensional (1D) and two-dimensional (2D) versions with the latter being mainly significant in EP.

1D separations are divided into separate parallel tracks, each of which represents the result of one sample run. Data from a particular track are acquired, processed and the results presented separately from those of all other tracks. 2D separations have to be acquired, processed and represented as a whole.

The separation as viewed by the densitometer represents an analog and (near) continuous picture. The information from all surface elements of the examined separation is available simultaneously, i.e. in parallel form. Processing is, however, in modern instruments virtually always done electronically with the input data organized in serial form. Before processing can begin, it is obviously necessary to convert the optical signal to an electrical one and to bring it into the form of a time varying one-dimensional signal. This process is commonly called scanning. Scanning can be performed on the optical side, before opto-electric conversion, or on the electrical side after conversion. This seemingly trivial difference has far reaching effects upon the design of the instrument and its operational features.

Processing of the acquired signal is by and large done in two steps: Preprocessing (or conditioning) of the acquired signal and final processing with the aim to extract the desired information, to separate it from other features and to present it to the operator in the most convenient form. In most modern instruments both steps are performed digitally on a computer [1]. For this purpose it is necessary to discretize the original analog signal and to bring it into digital form. The first operation is commonly called "sampling", the second "digitizing" proper.

Sampling rate and the number of digitizing steps must be chosen according to well established rules. The interested reader is here referred to literature [2]. Detailed discussion of these points here is thought to be beyond the scope of this paper.

TRADITIONAL SCANNING METHODS IN DENSITOMETRY

Traditional densitometers perform scanning by displacing the illuminating beam relatively to the scanned pattern by mechanical or mechano-optical means. The beam displacement is generally arranged in such a way, that the illuminated aperture follows a sequence of closely spaced parallel lines. This scanning mode, widely used also in other fields, e.g. in T.V., computer monitors etc., is called "raster scanning". In 1D densitometry two types of raster scanning are commonly employed. (Fig. 1). They differ by the profiles of the scanning aperture, which can be either point-shaped or rectangular [3].

Functionally most flexible is the first one which leads to a scanning mode generally called "flying spot" or "point" scanning. The term "meander scanning" is used, when the beam displacement follows an approximate zig-zag pattern (Fig. 2). The illuminated area can have circular or quadratic shape. The result of the scanning process is an analog signal, which represents the spatial distribution of the optical density along the scanned line as a function of time. For subsequent computer processing is the originally continuous signal discretized into continuous short segments; the amplitude of each segment is then digitized and fed to the processing computer.

Instead of continuous displacement of the scanning beam a stepping motion is sometimes used. It is functionally superior, but mechanically more difficult to implement. Discretization is here an intrinsic consequence of the step-displacement of the beam. The length of scan line is with 1D separations usually made equal to the width of the individual track. Only some types of electronic scanning systems use also for 1D, i.e. for multitrack separations, scan widths which extend over several tracks or even the whole width of the separating medium. The acquired data are then in software assigned to the respective track areas.

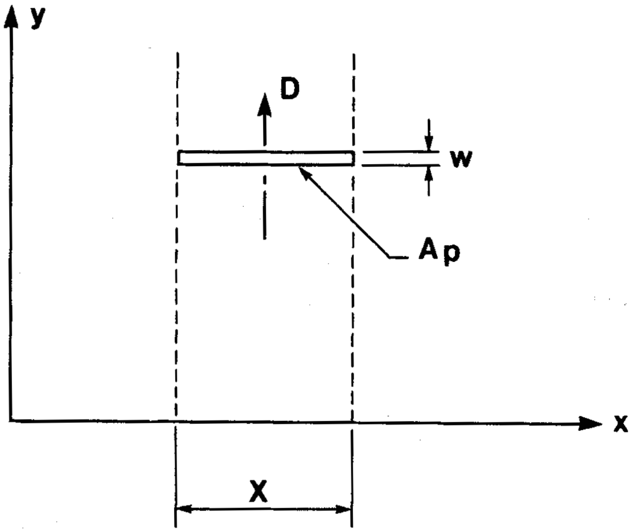

Figure 1a. Slit scanning.

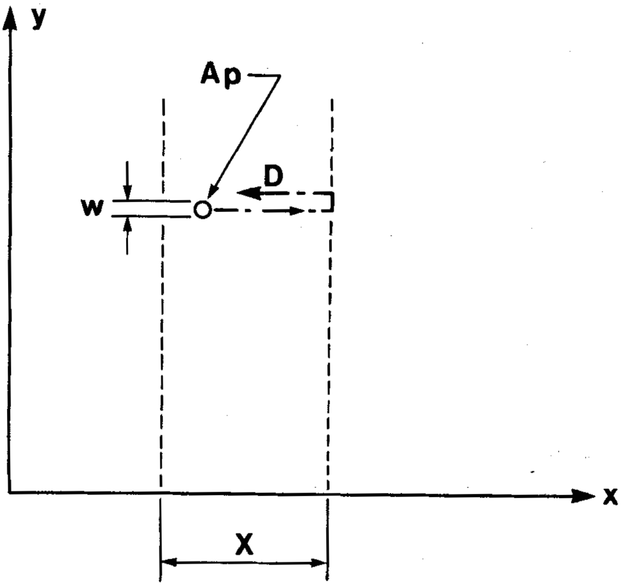

Figure 1b. Point scanning
w = width of aperture A_p X width of track
D = direction of scan

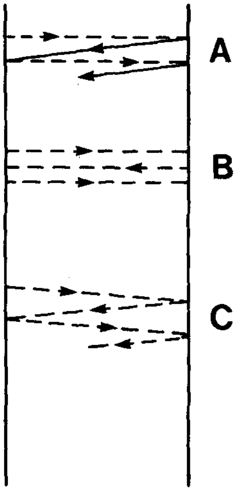

Figure 2 A. Straight raster scan; fly back is blanked out
B. Meander scan; fly back active, but in reverse direction

A rectangular profile of the scanning beam leads to a different mode of scanning, for which the term "slit scanning" is in use. The width of the scanning beam is here made equal to the width of the scanned track. It follows, that slit scanning is not very useful for 2D separations which are not divided into tracks. The information contained in an individual scan line is here acquired as a whole and not as a sequence of separate point data. That scanning is extensively used in today's densitometers for the analysis of 1D separations. The advantages and disadvantages of point scanning and slit scanning are on a comparative basis discussed in [4].

Existing densitometers for 1D separations and some for 2D ones still use almost exclusively opto-mechanical scanning; conversion to electrical form takes place after the scanning process. The approach was virtually the only one compatible with the technological level of its time. The only photo-electric converter which was able to work under the given optical conditions with adequate signal to noise ratio was the photomultiplier (PM) tube. The principal advantages are sensitivity and signal to noise ratio which are hardly matched by that of any other photodetector. Another important advantage is, that PM tubes can be equipped with a wide range of phosphors with different spectral sensitivities. Besides these advantages there are, however, also drawbacks, which have lead to a search for other types of photosensors for densitometry. One of these drawbacks is the relatively large physical size of the PM tube, which prevents its use in multiple detector assemblies. (Two detector elements in simultaneous operation seem to be the practical limit). It took many years of development before sensitivity and spectral response of the much smaller solid state photodiodes reached levels, where their use in lieu of PM tubes became feasible. But the physical dimensions of this type of detector were still too large to permit their use in large multidetector arrays.

MODES OF IMPLEMENTATION OF OPTO-MECHANICAL SCANNING

Optical scanning is in principle implemented in one of two ways: a collimated scanning beam shaped by a point or slit shaped aperture is deflected in the direction of the scan lines and (in the first case) perpendicularly to them by an oscillating system of plane mirrors. Alternatively revolving polygonal reflecting prisms can be used. Combinations of the two mechanisms with oscillating mirrors in one direction and revolving prisms in the other are also found. The oscillating and/or revolving motions are controlled by a time base with synchronizes them with the process of sampling and digitization.

Another and in some respects superior alternative is to keep the illuminating beam stationary and to move instead the support table with the medium in the direction of the lines and columns of the raster. The displacement can be continuous or step-wise. There is no decisive advantage for either version. It may seem paradoxical, but displacement of the support with the required rigid control of the motion of relatively large masses leads generally to a design, which is both mechanically and optically easier to implement than the moving beam option. Its main advantage is, that projecting the image of the stationary scanning aperture upon the photodetector is simpler and easier than the same task with a moving aperture.

Optical scanning by either method works with relatively small apertures. Concentration of the available light flux upon this area and its uniform illumination is easy and efficient.

Spectral sensitivity using a monochromator is also easy to implement and with modern high intensity light sources and suitable design can the intensity of the light reaching the photodetector be made adequate for the operation of present high sensitivity solid state devices, mostly photodiodes of different types. The method if properly executed yields instruments with high sensitivity, good spatial resolution and a dynamic range of several OD (optical density) units.

The principal disadvantage of densitometers with opto-mechanical scanning is expensive design and slow operating speed. The time required for scanning a separation of given size increases, other things equal, approx. in proportion with the square of the required resolution.

LASER SCANNING

The spatial resolution which can be obtained with traditional opto-mechanical scanning is adequate for most densitometric applications in TLC and EP. An exception are some types of 2D electrophoretic separations, where an extremely high spatial resolution is some times desired. A resolution of a few microns can be obtained, when a laser is used as light source [5]. The beam of coherent light produced by a laser can be collimated to much smaller diameters than that of a conventional light source. Displacement of the laser beam with respect to the scanned separation is performed in the same way as with a conventional light source.

The high resolution obtainable by laser scanning increases obviously the time needed to scan a pattern of given size. The high energy density of the laser beam permits higher scanning speeds [6]. The gain in speed can, however, not always outweigh the increased acquisition time due to the much larger number of data points. Care is also necessary to avoid photochemical decomposition of the separated material.

It must be noted that laser light has a fixed wave length. Laser scanning is, therefore, impractical where the possibility to change the spectrum of the illuminating is desired. Lasers with adjustable wave length are available. For the time being they are too costly to be used in commercial instruments.

Spatial resolutions approaching that of a laser scan can be obtained by linear arrays of photodiodes with mechanical advance in the direction of flow.

PHOTOACOUSTIC SENSORS

A rather unorthodox type of image sensor has recently made its debut in the field of 2D densitometry. It seems to be less suitable for 1D measurements. The device is generally known as photo-acoustic light sensor [7]. It originated in the field of non-dispersive spectral analysis principally for measurements in the infrared. It remains to be seen, to what extent it will prove itself in densitometry. The working principle of the device is the pressure increase of a small volume of gas when exposed to radiation reflected from a small area segment of the scanned surface. The pressure change which is proportional to the intensity of the reflected radiation is sensed by a highly sensitive microphone. The illuminating beam is interrupted (chopped) at an acoustic or subacoustic frequency. The output of the sensing microphone has then the same frequency, a feature which gave the device its name. By suitably choosing the gas filling of the measuring volume the sensor can be tuned to a specific wave length of the illuminating wave length over a wide range. Scanning is done by mechanically displacing the position of the sensor with respect to the scanned medium. Actual performance data do not yet seem to be available. It also appears that it has not yet been incorporated into- a commercial instrument.

ELECTRONIC SCANNING

The characteristic feature of electronic scanning is, that the conversion of the image data to serial form is performed on the signal already in electrical form. Multi-element photodetector arrays are necessary for that [8].

Detectors of this type can be divided into (vacuum) tube sensors and solid state devices. The latter can be subdivided into line and area (matrix) sensors. Linear vacuum sensors do not seem to be commercially available.

Sensors of the matrix type are also called image sensors proper, because all elements of the scanned object are converted to electrical form simultaneously similarly as the eye translates all elements of an observed picture simultaneously to nervous impulses. Line scanners do the same, but only with elements of a single scan line. Successive lines are scanned sequentially; the necessary shift of the field of view of the sensor is realized by similar technics as used for the scan displacement with optical scanning.

The simultaneous acquisition of pixel data from a scan line or a viewed surface requires an illuminating system, which is able to provide uniform light intensity over the length of a scan line or surface respectively. Fairly sophisticated optical systems are necessary to achieve that. Minor deviations from uniformity, since they do not change with time, can be corrected in software. Spreading the available light energy over a line or area reduces, of course, significantly the energy per pixel. This becomes especially serious, where spectrally narrow illumination is desired, e.g. for the scanning of substances with narrow absorbance spectrum. Selection of a narrow spectral band from a broad band light source entails obviously a large loss of energy. The remaining light energy when spread over a large surface may than not be sufficient to guarantee an adequate signal to noise ratio of the received signal. A high intensity light source and a sensitive photo-detector array are necessary to cope with the problem.

Most present days solid state image sensing arrays have poor sensitivity in the UV. Work to improve UV response is in progress, but is slow because fundamental physical problems must be resolved. Indirect techniques using fluorescence or fluorescence quenching seem to be presently the best expedient [9].

On the other hand most array sensors, especially those of the semiconductor type can be produced with extended sensitivity in the infrared. This may open an up to now in densitometry little used spectral range for analysis purposes.

The design of a densitometer with electronic scanning is for comparable performance simpler and less expensive than that of an instrument with optical scanning. Another significant advantage is the much higher speed of data acquisition, which with matrix (2D) scanners can be as low as 25 to 30 ms. Since the objects used in densitometry are without exception stationary, multiple scanning can be employed to improve sensitivity and signal to noise ratio.

Spatial resolution of present days matrix sensors is comparable and with some types of scanners superior to that of most opto-mechanical scanners. The resolution, which can be obtained with some solid state line scanners approaches that of laser scanners.

VACUUM IMAGE SENSORS

Vacuum image sensors frequently also called camera tubes are the oldest but still widely used type of image sensors. Of the many different types available mainly the vidicon and its many relatives, e.g. the plumbicon, saticon, newicon, etc., are for densitometry of importance. They have undergone significant improvement and are today available at moderate cost and with good sensitivity and spectral range. Special versions exist with spatial resolution in excess of 2000 lines. Tubes with UV and even X-ray sensitivity are also on the market [10].

The image projected onto the face plate of the tube is scanned by an electronic beam generally at the TV rate of 25 or 30 ms per passage. The scanning motion of the beam is produced by time varying magnetic or electric fields. It follows immediately that the tube is sensitive to external fields of the respective type. *It is also difficult to make the (spatially defined) scanning* speed absolutely uniform over the whole photosensitive area of the face plate. The scanning speed should also remain unchanged over prolonged periods of time. Long strides have been made towards these goals, but deviations still exist. Geometric distortion and instability are the immediate consequences but can for general purpose devices be kept in acceptable limits.

The dynamic range of the output signal is for linear response rarely better than about 1:100. Excessive light intensities may cause temporary and even permanent damage to the tube. The operating sensitivity depends upon the type of the tube and the wave length of the illuminating light. As a rough estimate it can be assumed that for blank picture elements an illumination density of about 100 mW/cm^2 is desirable. The measurable absorbance range for separated substance can then be expected to be of the order of 2 OD (optical density) units. Special tubes can work at even lower light levels and cover a larger dynamic range. In the extreme the use of a high resolution image intensifier can be considered.

SOLID STATE SENSORS

Considered here are only multi-elements sensors in large scale integrated (LSI) design [11], [12]. They exist, as already remarked, in linear (1D) form and as matrix devices. LSI design implies that the sensing elements, usually photodiodes, are produced together with the necessary read-out circuitry on a single chip of semiconductor material. The yield in mass production of near perfect devices, which are necessary for densitometric applications, declines rapidly, when the density of components on the chip increases, and cost and production difficulties go up in proportion. If for a linear sensor a certain spatial resolution can be achieved with N elements, a matrix sensor with similar resolution requires about N^2 elements. This explains why linear sensors can achieve a higher spatial resolution than matrix devices. The former are currently available with up to about 2000 (and some high priced models with about 4000) independent photodiodes. Routine high resolution matrix sensors have at today's technology up to 700 x 600 diodes [13], [14]. Technology improvements can be expected to increase the sensor density in the near future still further. As example for what is already today for special purposes possible can be quoted the CCD scientific (matrix) sensor TK 2048M of Tektronix, Beaverton, Oregon, USA with 2048 x 2048 diodes, quoted to be 10 to 100 times more sensitive than conventional high speed photographic film.

Most solid state sensors are built on the basis of silicon with "charge coupling" (CCD) and its relatives for read out [15]. The physical properties of silicon make extension of the spectral sensitivity into the UV difficult. Nonetheless are devices with good UV sensitivity (approaching 200nm), though with lower resolution, already on the market and further improvements can be expected soon [16], [17].

Noteworthy in the spectral regard are pyro-electric sensors, which are built from different material and use a different physical principle [18]. The sensing element is here in essence a subminiature temperature sensor, which measures the local energy density of the incident radiation. It has, therefore, a flat spectral characteristic ranging from the deep UV to the far IR. Its draw back is a sensitivity which is several orders of magnitude lower than that of good photodiodes. The main problem with their use in densitometry is the difficulty to obtain with present light sources an adequate energy density over a narrow spectral range.

SOLID STATE IMAGE SENSOR VERSUS CAMERA TUBES

Solid state sensors are at today's level of technological development more expensive than vacuum devices with comparable performance. Nonetheless the trend goes towards increasing use of solid state elements. A comparison of the relative advantages of the two types of sensors is, therefore, in place. It is restricted to matrix devices, because linear vacuum sensors do not seem to exist on a commercial scale.

Solid state sensors in LSI design are physically small and rugged. There is no electron scanning beam and they are, therefore, insensitive to external magnetic or electric fields. They are geometrically stable and free of spatial distortion, because these features are here determined by the geometry of the design, which can be made very precise and is temporally stable. The sensors can handle linearly a much larger dynamic range than vidicon tubes and even severe over-exposure cannot damage the device. At worst it can produce temporary overload of the most affected diodes. Also aging problems are, compared to tubes, nearly absent.

On the negative side there is to mention the more limited spectral range of most solid state devices in the UV, which is only partly outweighed by an extended IR range. Solid state devices are also temperature sensitive; for instruments to be used indoors at near constant ambient temperature this is probably only a minor consideration.

APPLICATION COMMENTS

It was already mentioned that uniform density of illumination becomes the more difficult to achieve, the larger the area to be illuminated. A similar effect as non-uniform illumination density has non-uniform sensitivity of the detector array. Even almost dead (insensitive) diodes occur sporadically also in the highest quality devices. The effects of these factors are generally designated as "fixed pattern noise". Since it is temporally stable, it is usually no problem to take care of it in software after data acquisition.

For best signal to noise ratio it is desirable to limit the spectral energy distribution of the illuminating light to a shape which closely equals the shape of the absorbance characteristic of the examined substance. When the latter is very sharp, the danger of excessive light loss may arise which can not always be compensated by increasing the power of the light source. This danger is small when the analyzed material has a broad band (gray) absorbance spectrum.

ANALOG TO DIGITAL CONVERSION AND DATA STORAGE

The data acquisition rate of electronic scanners is extremely high. Correspondingly fast sampling and analog to digital (A/D) converters are, therefore, necessary. The converted data are then stored in a correspondingly large intermediate digital memory, from where they can be shifted to the processing unit or to long term storage on magnetic disk or tape.

The development of fast "flash" converters and the associate storage circuitry has succeeded and the devices are available at moderate cost, in part as add-on boards, which can be operated with any of the modern powerful microcomputers. The basic elements are usually 8 bit converters and a 512 x 512 x 8 bit picture memory with associated circuitry. Converters with 10 and even 12 bit amplitude resolution are available, but their higher cost is warranted only for top of the line devices. Similarly picture memories with higher storage capacity, e.g. 1024 x 1024 x 8 (or more) are equally available at correspondingly higher cost.

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